tion of iron metabolism in eukaryotes is multifaceted and involves transcriptional, posttranscriptional, and posttranslational mechanisms.

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Selected reading

Jing, Q., Huang, S., Guth, S., Zarubin, T., Motoyama, A., Chen, J., Di Padova, F., Lin, S.C., Gram, H., and Han, J. (2005). Cell *120*, 623–634.

Kim, M., Krogan, N.J., Vasiljeva, L., Rando, O.J., Nedea, E., Greenblatt, J.F., and Buratowski, S. (2004). Nature *432*, 517–522.

Lee, A., Henras, A.K., and Chanfreau, G. (2005). Mol. Cell *19*, 39–51.

Lykke-Andersen, J., and Wagner, E. (2005). Genes Dev. *19*, 351–361.

Puig, S., Askeland, E., and Thiele, D.J. (2005). Cell *120*, 99–110.

Shakoury-Elizeh, M., Tiedeman, J., Rashford, J., Ferea, T., Demeter, J., Garcia, E., Rolfes, R., Brown, P.O., Botstein, D., and Philpott, C.C. (2004). Mol. Biol. Cell *15*, 1233–1243.

Wyers, F., Rougemaille, M., Badis, G., Rousselle, J.C., Dufour, M.E., Boulay, J., Regnault, B., Devaux, F., Namane, A., Seraphin, B., et al. (2005). Cell *121*, 725–737.

Yamaguchi-Iwai, Y., Ueta, R., Fukunaka, A., and Sasaki, R. (2002). J. Biol. Chem. *277*, 18914–18919.

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When pouring water on the fire makes it burn brighter

Earlier work seems to suggest that overexpression of uncoupling protein 1 would be anti-inflammatory. However, new findings show that expression of uncoupling protein 1 in aortic smooth muscle cells of mice increases reactive oxygen species, activates the renin-angiotensin system, elevates blood pressure, and worsens atherosclerosis.

Uncoupling proteins are inner mitochondrial membrane anion transporters that allow protons to leak back into the mitochondrial matrix and decrease the energy available for ADP phosphorylation. Less ADP phosphorylation should lead to the production of less reactive oxygen species (ROS). Thus, Semenkovich and colleagues (Bernal-Mizrachi et al., 2005) expected that the overexpression of uncoupling protein 1 (UCP1) in mice would lead to decreased ROS. Surprisingly, they found that doxycycline-inducible expression of UCP1, restricted to vascular smooth muscle cells by use of the SM22α promoter, resulted in markedly increased levels of aortic superoxide and nitrotyrosine. Transgenic expression of UCP1 increased peroxynitrite production due to the interaction of superoxide with nitric oxide in the aorta. As a result, blood pressure (as measured by noninvasive tail cuff as well as with implanted telemetry units) was significantly increased. The authors also found that atherosclerosis, measured by en face analysis, was increased on feeding a high-cholesterol high-fat diet (Western diet) to transgene-bearing mice that were crossbred to also lack apolipoprotein (apo) E. When these mice were fed a chow diet, blood pressure but not aortic atherosclerosis was increased; the authors concluded that increased blood pressure was not the cause of the increased atherosclerosis. Plasma lipoprotein profiles were also not increased in the mice expressing vascular UCP1. The mice did show a significant increase in plasma renin activity and a decrease in urinary sodium excretion, confirming activation of the renin-angiotensin system.

While the increase in ROS resulting from the overexpression of UCP1 was unexpected, the subsequent increase in atherosclerosis after crossbreeding the transgene into apoE null mice fed a Western diet was not. The coupling of ROS and lipids in the artery wall has been shown to increase atherosclerosis in a number of mouse models. The binding of lipoproteins containing apoB in the artery wall is common in all species that develop atherosclerosis. ApoB has an amino acid sequence that binds to extracellular matrix proteoglycans found in the subendothelial space of arteries. As a result, the concentration of lipoproteins containing apoB (e.g., low-density lipoproteins [LDL]) in artery walls is twice that in the circulation, even in normal arteries. This is in contrast to apoA-I, the major protein in high-density lipoproteins (HDL). The concentration of apoA-I in normal arteries is far less than the concentration in the circulation.

Pioneering studies, done more than 100 years ago, demonstrated that rabbits fed a high-cholesterol diet develop atherosclerosis; subsequent epidemiologic studies have correlated LDL cholesterol levels with risk for atherosclerosis. Because of these findings, early work focused on the role of cholesterol in the apoB-containing lipoproteins. These lipoproteins are not only rich in cholesterol but are also very rich in phospholipids, which contain polyunsaturated fatty acids, particularly arachidonic acid and linoleic acid. These phospholipids are highly susceptible to free radical oxidation. As shown in Figure 1, the resulting oxidized phospholipids induce the artery wall cells to produce potent monocyte chemoattractants such as monocyte chemoattractant-1 (MCP-1) and also cause the artery wall cells to produce monocyte differentiation factors such as monocyte colony stimulating factor (M-CSF) (Navab et al., 2004). A number of different ROS can attack arachidonic and linoleic acids, resulting in the formation of the same proinflammatory oxidized phospholipids. Once formed, these proinflammatory lipids evoke the same inflammatory response, regardless of the specific ROS that produced them. ROS derived from a variety of cell types and pathways in the artery wall have

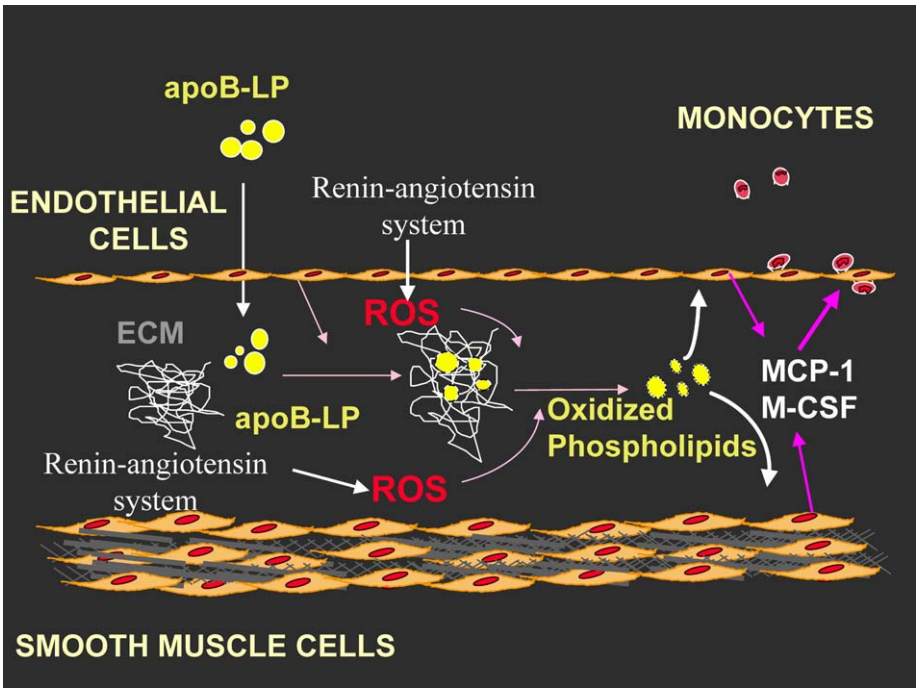

Figure 1. Oxidized phospholipids induce artery wall inflammation

Lipoproteins (LP) containing apoB (apoB-LP) are trapped in the subendothelial space of arteries because they bind to the extracellular matrix proteins (ECM). Reactive oxygen species (ROS) derived from multiple pathways in the artery wall (e.g., conditional overexpression of uncoupling protein 1, NADPH oxidase pathway, 12/15-lipoxygenase pathway, 5-lipoxygenase pathway, myeloperoxidase pathway) oxidize apoB-LP phospholipids, which contain arachidonic acid or linoleic acid and generate pro-inflammatory oxidized phospholipids. These oxidized phospholipids stimulate the production of monocyte chemoattactants such as MCP-1 and monocyte differentiation factors such as M-CSF. This causes the monocytes to migrate into the artery wall and differentiate into macrophages. The renin-angiotensin system enhances the production of ROS and provides a positive feedback loop to amplify the inflammation.

been shown to increase atherosclerosis in mouse models and/or have been found associated with human atherosclerotic lesions. ROS derived from the NADPH oxidase pathway (Cathcart 2004), the 12/15-lipoxygenase pathway (Huo et al., 2004), the 5-lipoxygenase pathway (Mehrabian et al., 2002), and the myeloperoxidase pathway (Nicholls and Hazen, 2005) have all been shown to increase atherosclerosis in mouse models and/or have been found associated with human atherosclerotic lesions. The oxidized phospholipids resulting from free radical oxidation evoke an inflammatory reaction in the artery wall similar to that seen in tissues infected with *Mycobacteria* (Navab et al., 2004). This inflammatory reaction, which is rich in monocyte macrophages and poor in neutrophils, was seen in the UCP1-overexpressing apoE null mice (Bernal-Mizrachi et al., 2005).

The activity of some of the ROS-generating and oxidized phospholipid-producing pathways is significantly increased by the renin-angiotensin pathway (see Figure 1 and Brasier et al. [2002]). The renin-angiotensin pathway was also markedly upregulated by the overexpression of UCP1 (see above). Angiotensin II, a product of the renin-angiotensin pathway, can increase the production of ROS, inflammatory cytokines, and adhesion molecules in the artery wall. Additionally, the aortic smooth muscle cell hypertrophy that results from angiotensin II-induced hypertension requires the recruitment of monocyte-macrophages (Bush et al., 2000), which was the major cell type in the atherosclerotic lesions described by Semenkovich and colleagues (Bernal-Mizrachi et al., 2005). This further links ROS, hypertension, and inflammation with atherosclerosis.

Currently, the major focus for preventing and treating atherosclerosis is reducing plasma LDL cholesterol levels. Semenkovich and colleagues (Bernal-Mizrachi et al., 2005) noted that the changes they observed were independent of changes in plasma lipoprotein profiles, and the authors suggested that therapies independent of lipid lowering might be effective in the prevention and treatment of atherosclerosis. It is interesting to note that, in apoE null mice, oral treatment with an apoA-I mimetic peptide (D-4F) resulted in dramatic reductions in atherosclerosis that were independent of changes in plasma lipids but were associated with decreased ROS (Navab et al., 2005a). This effect was synergistic with the non-lipid-lowering actions of statins (Navab et al., 2005b). These results lend support to Semenkovich and colleagues' (Bernal-Mizrachi et al., 2005) suggestion that treatments directed at reducing ROS independent of lipid lowering may be beneficial in the prevention and treatment of atherosclerosis.

The recent studies by Semenkovich and colleagues (Bernal-Mizrachi et al., 2005) originated with the serendipitous finding that overexpressing UCP1 in smooth muscle cells unexpectedly increased ROS instead of causing the predicted decrease. The mechanisms by which ROS were increased, rather than decreased, remain to be determined. Studies directed at discovering these mechanisms are likely to reveal new insights into mitochondrial structure and function. Regardless of the mechanisms, the potential importance of mitochondria as a source of ROS that can participate in both hypertension and atherosclerosis is clearly highlighted by these studies (Bernal-Mizrachi et al., 2005). It will be interesting and potentially important to determine if the benefits of the non-lipid-lowering properties of statins and apoA-I mimetic peptides such as D-4F are in part mediated by an action on mitochondrial ROS.

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Selected reading

Bernal-Mizrachi, C., Gates, A.C., Weng, S., Imamura, T., Knutsen, R.H., DeSantis, P., Coleman, T., Townsend, R.R., Muglila, L.J., and Semenkovich, C.F. (2005). Nature *435*, 502–506.

Brasier, A.R., Recinoss, A., and Eledrisi, M.S. (2002). Arterioscler. Thromb. Vasc. Biol. *22*, 1257–1266.

Bush, E., Maeda, N., Kuziel, W.A., Dawson, T.C., Wilcox, J.N., DeLeon, H., and Taylor, R. (2000). Hypertension *36*, 360–363.

Cathcart, M.K. (2004). Arterioscler. Thromb. Vasc. Biol. *24*, 23–28.

Huo, Y., Zhao, L., Hyman, M.C., Shashkin, P., Harry, B.L., Burcin, T., Forlow, B., Startk, M.A., Smith, D.F., Clarke, S., et al. (2004). Circulation *110*, 2024–2031.

Mehrabian, M., Allayee, H., Wong, J., Shih, W., Wang, X.-P., Shaposhnik, Z., Funk, C.D., and Lusis, A.J. (2002). Circ. Res. *91*, 120–126.

Navab, M., Anantharamaiah, G.M., Reddy, S.T., Van Lenten, B.J., Ansell, B.J., Fonarow, G.C., Vahabzadeh, K., Hama, S., Hough, G., Kamranpour, N., et al. (2004). J. Lipid Res. *45*, 993–1007.

Navab, M., Anantharamaiah, G.M., Reddy, S.T., Hama, S., Hough, G., Grijalva, V.R., Yu, N., Ansell, B.J., Datta, G., Garber, D.W., and Fogelman, A.M. (2005a). Arterioscler. Thromb. Vasc. Biol. *25*, 1–7.

Navab, M., Anantharamaiah, G.M., Hama, S., Hough, G., Reddy, S.T., Frank, J.S., Garber, D.W., Handattu, S., and Fogelman, A.M. (2005b). Arterioscler. Thromb. Vasc. Biol. *25*, 1–7.

Nicholls, S.J., and Hazen, S.L. (2005). Arterioscler. Thromb. Vasc. Biol. *25*, 1102–1111.

DOI 10.1016/j.cmet.2005.06.009